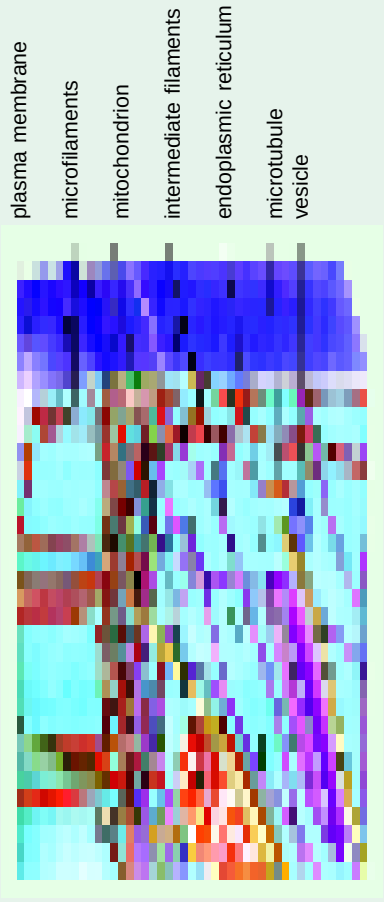
CHAPTER

15

Cytoskeleton : Microtubules, Microfilaments and Intermediate Filaments



Eukaryotic cells are given shape and organization by the cytoskeleton, which consists of three types of proteins: microtubules, intermediate filaments, and microfilaments.

The ability of eukaryotic cells to adopt a variety of shapes and to carry out coordinated and directed movements depends on the **cytoskeleton**. The cytoskeleton extends throughout the cytoplasm and is a complex network of three types of protein filaments : **microtubules**, **microfilaments** (or actinfilaments) and **intermediate filaments** (**IFs**). The cytoskeleton is also can be referred to as **cytomusculature**, because, it is directly involved in movements such as crawling of cells on a substratum, muscle contraction and the many changes in the shape of a developing vertebrate embryo; it also provides the machinery for the cyclosis in cytoplasm. Cytoskeleton is apparently absent from the bacteria; it may have been a significant factor in the evolution of the eukaryotic cells.

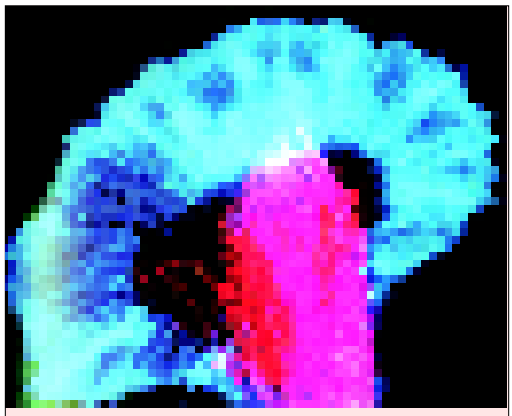
The existence of an organized fibrous array or cytoskeleton in the structure of the protoplasm was postulated in 1928 by **Koltzoff**. He conceived of a cytoskeleton that determines both the shape of the cell and the changes in its form.

The main proteins that are present in the cytoskeleton are **tubulin** (in the microtubules),**actin**, **myosin**, **tropomyosin** and other (in the microfilaments) and **keratins**, **vimentin**,

desmin, **lamin** and others (in intermediate filaments). Tubulin and actin are globular proteins, while subunits of intermediate filaments are fibrous proteins. Great progress has been made in the isolation of these cytoskeletal proteins. In addition, by the production of specific antibodies against these proteins, it has been possible to examine under the light and the electron microscopes the disposition of the microtubules and microfilaments. The use of high-voltage electron microscopy on whole cells has also helped to demonstrate that there is a highly structured, three-dimensional lattice in the ground cytoplasm.

MICROTUBULES

Microtubules were fisrt of all observed in the axoplasm of the myelinated nerve fibres by **Robertis** and **Franchi** (1953). They called them **neurotubules**. The exact nature of microtubules was brought into light when **Sabatini**, **Bensch** and **Barnett** (1963) made use of the glutarldehyde fixative in the electron microscopy. Microtubeles of plant cells were first described in detail by **Ledbetter** and **Porter** (1963).



The terminus of a growing axon. Actin filaments are shown in blue, microtubules in red.

Occurrence

With rare exceptions such as the human erythrocytes, microtubules are found in all eukaryotic cells, either free in the cytoplasm or forming part of centrioles, cilia and flagella. The most abundant source of microtubules for the biochemical studies is vertebrate brain— high densities of microtubules exist in axons and dendrites of nerve cells. In the cytoplasm of animal and plant cells, microtubules occur at following seven sites :— 1. cilia and flagella, 2. centrioles and basal bodies, 3. nerve processes, 4. the mitotic apparatus, 5. the cortex of meristematic plant cells, 6. elongating cells such as during the formation of the lens or during spermatogenesis of certain insects. 7. selected structures in Protozoa such as the axostyle of parasitic flagellates, the axoneme of *Echinosphaerium*, the fibre systems of *Stentor*, and the cytopharyngeal basket of *Nassula*.

The stability of different microtubules varies. Cytoplasmic and spindle microtubules are rather labile structures, whereas, those of cilia and flagella are more resistant to various treatments.

Structure

Microtubules constitute a class of morphologically and chemically related filamentous rods which are common to both plant and animal cells. A microtubule consists of a long, unbranched, hollow tubules 24–25 nm in diameter, several micrometers long and with 6 nm thick wall having 13 subunits or **protofilaments**. Thus, the wall of the microtubule consists of 13 individual linear or spiralling filamentous structures about 5 nm in diameter, which in turn, are composed of tubulin. These protofilaments have a centre-to-centre spacing of 4.5 nm. Application of negative staining techniques has shown that microtubules have a lumen 14 nm wide and a protofilament or subunit structure in the wall (Fig. 15.1).

Chemical Composition

Biochemically, a protofilament of microtubule is made of a protein called **tubulin**. Tubulin is an acidic protein with a molecular weight of 55,000 and a sedimentation constant of 6S. It occurs in two different forms, called **α-tubulin** and **β-tubulin**, each containing about 450 amino acids. Both of these proteins have a distinct, though closely related, amino acid sequences and are thought to have evolved from a single ancestral protein. The two proteins show very little divergence from the lowest to the

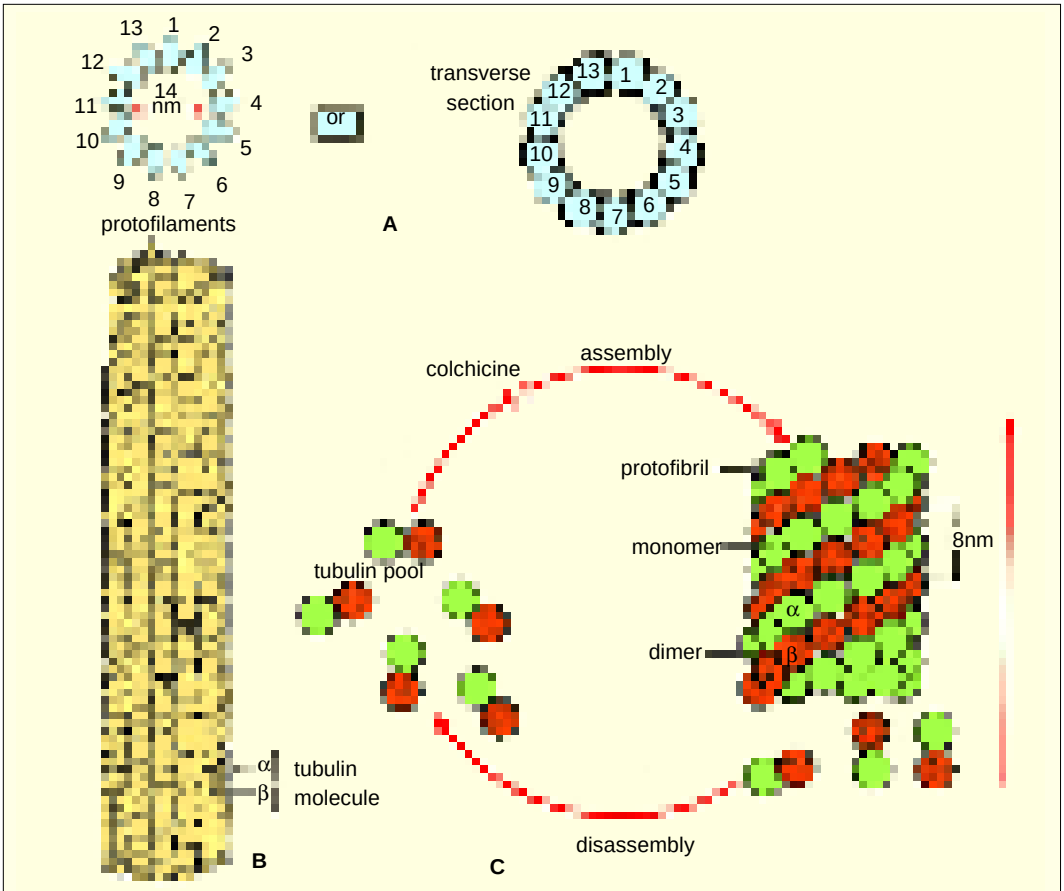


Fig. 15.1. Schematic diagrams of a microtubule, showing how the tubulin molecules pack together to formthe cylindrical wall. A—13 tubulin molecules (subunits of protofilaments) in cross section ; B—Side view of a short section of a microtubule, with the tubulin molecules aligned into rows, or protofilaments. Each of the 13 protofilaments is composed of a series of tubulin molecules, each with an α/β heterodimer ; C—Assembly and disassembly of the microtubule. The microtubule is being disassembled at the bottom while being simultaneoasly assembled at the top. Colchicine, by blocking the assembly process, produces depolymerization of the microtubules (after De Robertis and De Robertis, Jr., 1987 ; Alberts *et al.*, 1989).

highest eukaryotes ; for example, the β- tubulins of sea urchin flagella and chick brain cells differ only in one amino acid. Similarities such as this suggest that most mutations disrupt the functions of microtubules and are thus lethal and are eliminated by selection (see **King**, 1986).

Tubulin in the form of dimers (rather heterodimers of α- and β- tubulins ; each with 115,000 MW, see **Berns**, 1983) polymerizes into the microtubules. Thus, heterodimers of tubulins assemble to form linear "protofilaments" with the β- tubulin of one dimer in contact with the α- tubulin of the next. Since all the 13 protofilaments are aligned parallely with the same polarity, the microtubules are the **polar** structurs having a **plus** or **fast growing end** and **minus** or **slow-growing end**. The minus ends of cytoplasmic microtubules in cells are bound tightly to **microtubule organizing centres** (**MTOCs**) from which their assembly or polymerization starts. MTOCs also protect the minus ends of the microtubules from the disassembly. Generally, the plus ends of microtubules terminate near cell margins (Fig. 15.2) and are protected from disassembly by the **capping proteins** (see **Alberts** *et al.*, 1989).

Microtubule-Associated Proteins (MAPs)

Recently, a number of proteins have been identified that associate with the surface of microtubules ; these proteins are called **microtubule-associated proteins** or **MAPs**. The following two major classes of MAPs have been isolated from brain in association with microtubules : 1. **HMW proteins** (=high molecular weight proteins) which have molecular weights of 200,000 to 300,000 or more ; 2. **tau proteins**, with molecular weights of 40,000 to 60,000. Both classes of proteins have two domains, one of which binds to microtubules ; because this domain binds to several unpolymerized tubulin molecules simultaneously, these MAPs tend to speed up the nucleation (= process of grouping around a central mass) step of tubulin polymerization *in vitro*. The other domain is believed to be involved in linking the microtubule to other cell components (Fig. 15.3). Antibodies to HMW and tau proteins show that both proteins bind along the entire length of cytoplasmic microtubules.

Microtubule Organizing Centres (MTOCs)

The microtubules are not found helter-skelter about the cell, but are organized in specific patterns designed to carry out specific function. Spontaneous nucleation, as seen *in vitro* (Fig. 15.1C), probably does not occur *in vivo*. Rather, initiation of assembly occurs at **microtubule organizing centres (MTOCs)**. Thus, MTOCs are nucleating centres that serve as templates for the polymerization of tubulin (see **Thorpe**, 1983). MTOCs exist in basal bodies (*e.g. Chlamydomonas*); in centrioles (*e.g.*, most animal cells); at the poles of mitotic spindles in dividing cells that do not have centrioles (*e.g.*, most plant cells); on chromosomes (*i.e.*, **kinetochore**); in membranes and probably many other places as well. Recent studies have revealed that most cytoplasmic microtubules do not arise directly from the centrioles, but from a densely staining **pericentriolar material** that surrounds the centriole (see **King**, 1986).

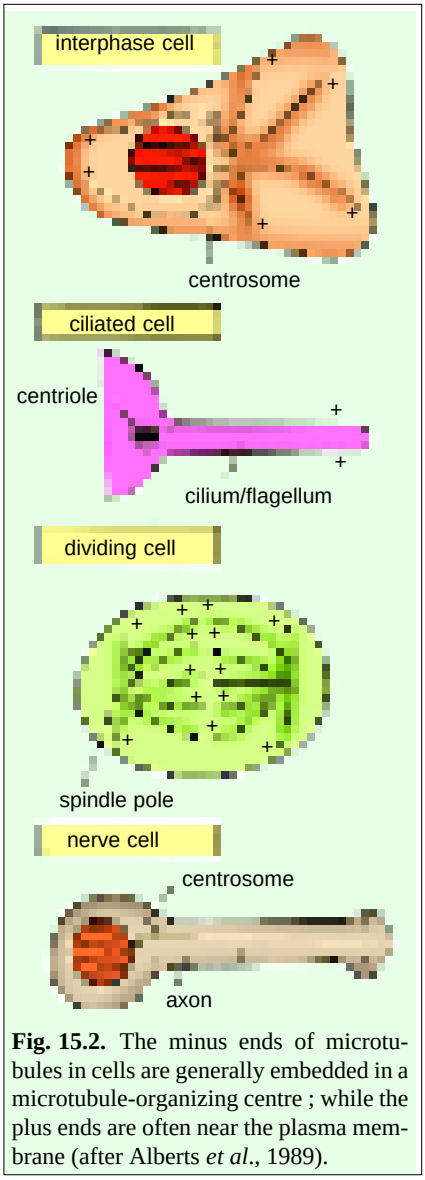


Fig. 15.2. The minus ends of microtubules in cells are generally embedded in a microtubule-organizing centre ; while the plus ends are often near the plasma membrane (after Alberts *et al.*, 1989).

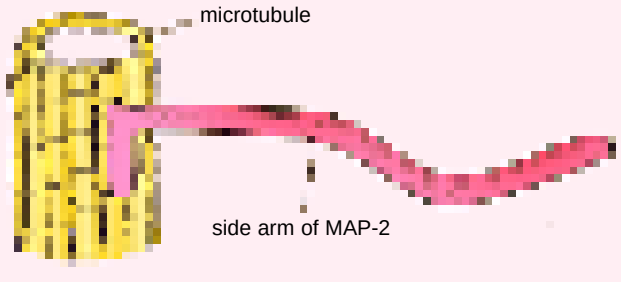
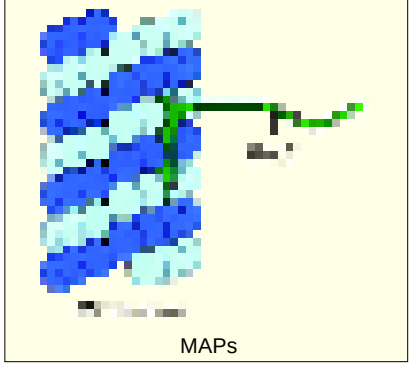


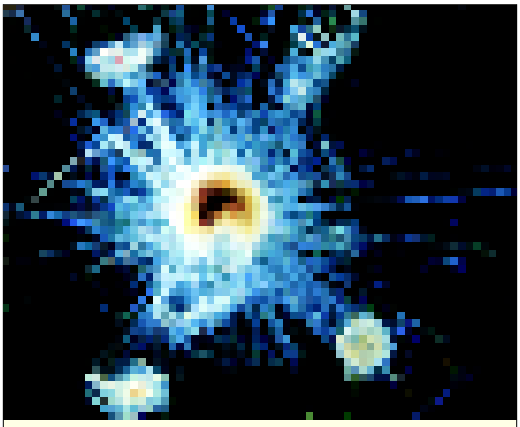
Fig. 15.3. A microtubule associated protein (known as MAP-2) showing its two domains (after Alberts *et al.*, 1989).



Turning on and off of these organizing centres for microtubule assembly at different times in the cell's life are probably regulated by one or all the following factors : changes in nucleation centres, changes in Ca^{2+} concentration and involvement of MAPs.

Assembly and Disassembly of Microtubules

Cytoplasmic microtubules are highly dynamic structures, constantly forming and disappearing depending on cell activities. They, like the microfilaments, grow by the reversible addition of subunits, accompanied by nucleotide (GTP) hydrolysis and conformational change. The process of polymerization (assembly) and depolymerization (disassembly) of the microtubules appears to be a form of **self-assembly**. The assembly of microtubules from the tubulin dimers is a specifically oriented and programmed process. In the cell, the sites of orientation are MTOCs from which the polymerization is directed. The quantity of polymerized tubulin is high at interphase (cytoplasmic microtubules) and metaphase (spindle microtubules), but low at prophase and anaphase.



Microtubules as supporting rods. This protist has tentacles projecting from its cell body.

Within the cell, microtubules are in equilibrium with free tubulin. Phosphorylation of the tubulin monomers by a cyclic AMP-dependent kinase favours the polymerization. A definite relationship has been found between cell shape, the number and direction of microtubules and cAMP. The assembly and disassembly of tubulin constitute a polarized phemomenon. In a microtubule, the assembly of tubulin dimers takes place at one end, while disassembly is common at the other end (Fig. 15.1 C). If a cell is treated with certain drugs such as **colchicine**, **vincristine** or **vinblastine**, the assembly of the microtubules is inhibited, while the disassembly continues, leading to the disorganization of the microtubule. Further, the assembly is accompanied by the hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP) and lack of GTP stops the assembly. *In vivo* control of assembly and disassembly of tubulin involves Ca^{2+} and the calcium-binding protein **calmodulin**. The addition of Ca^{2+} inhibits polymerization of tubulin ; this effect is also enhanced by the addition of calmodulin.

The *in vivo* mechanism involved in self-assembly of the microtubules is still little understood, however, *in vitro* studies have revealed various interesting facts about it. Thus, in a classical study using isolated bovine brain tubulin, **Weingarten** *et al.*, (1975) demonstrated that tubulin alone was not sufficient to bring about *in vitro* assembly into microtubules. Under normal conditions, if brain microtubules are isolated and caused to depolymerize into tubulin subunits, the tubulin molecules will reassemble into microtubules if Mg^{2+} and GTP (an energy source) are added to the mixture. However, according to **King** (1986), *in vitro* assembly of microtubules can occur in the presence of low calcium concentration, MAPs, GTP, and a level of free tubulin monomers above a threshold concentration.

In vitro polymerization evidently involves two distinct phases, one of **initiation** and the other of **elongation**. The initiation event seems to involve the formation of some multimeric "**nucleating**" centre, following which the addition of more subunits proceeds rapidly during elongation. Thus, during *in vitro* polymerization of microtubules, α- and β- tubulins combine to form heterodimers (Fig. 15.4). The heterodimers associate to form multimeric **rings**, **spirals** and other intermediate structures which eventually open up to form strands or protofilaments. Side-by-side assembly of the protofilaments creates sheet-like structures that curl to form a tube. Elongation of this short cylinder occurs by direct

addition of new heterodimers at one end of the tubule (*i.e.*, the plus end of tubule). It is believed that during anaphase, addition of dimers to one end of a microtubule is accompanied by the loss of dimers from the other end.

Functions of Cytoplasmic Microtubules

Microtubules have several functions in the eukaryotic cells such as follows :

1. Mechanical function. The shape of the cell (*e.g.*, red blood cells of non-mammalian vertebrates) and some cell processes or protuberances such as axons and dendrites of neurons, microvilli, etc., have been correlated to the orientation and distribution of microtubules.

2. Morphogenesis. During cell differentiation, the mechanical function of microtubules is used to determine the shape of the developing cells. For example, the enormous elongation in the nucleus of the spermatid during spermiogenesis is accompanied by the production of an orderly array of microtubules that are wrapped around the nucleus in a double helical arrangement. Likewise, the elongation of the cells during induction of the lens placode in the eye is also accompanied by the appearance of numerous microtubules.

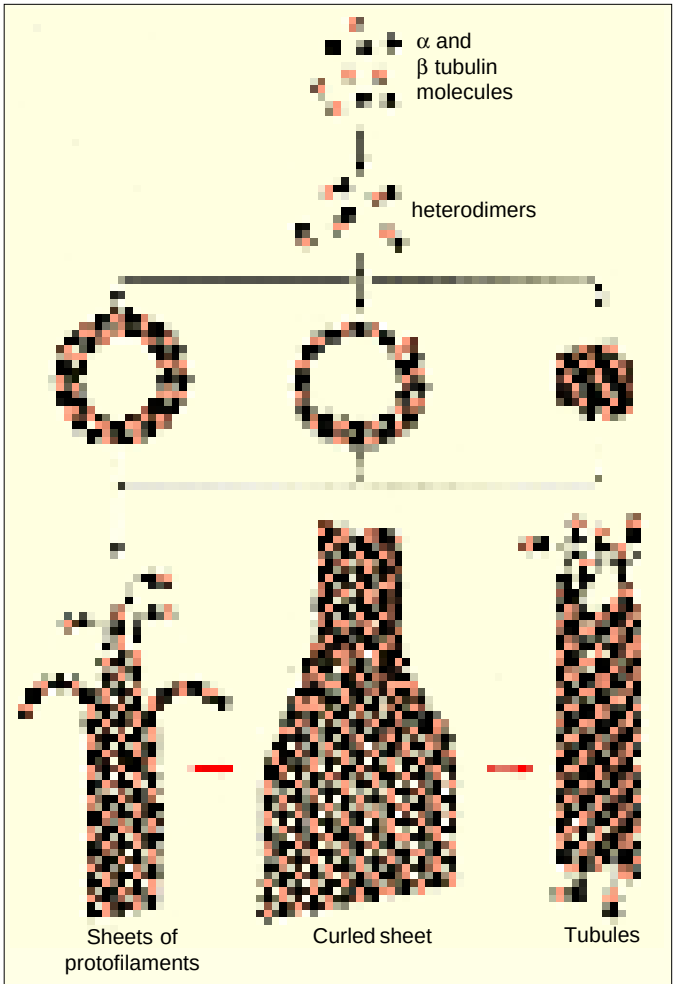


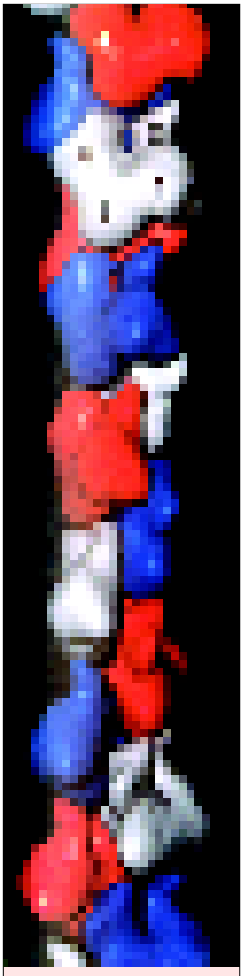
Fig. 15.4. Various steps in *in vitro* polymerization or assembly of the microtubules (After Thorpe, 1984).

This photo shows the nucleus (N) of a cell being held in place by a network of cytoskeleton.

3. Cellular polarity and motility. The determination of the intrinsic polarity of certain cells is also related to the microtubules. Directional gliding of cultured cells is found to depend on the microtubules.

4. Contraction. Microtubules play a role in the contraction of the spindle and movement of chromosomes and centrioles as well as in ciliary and flagellar motion.

5. Circulation and transport. Microtubules are involved in the transport of macromol-



Actin filament structure.

ecules, granules and vesicles within the cell. **Examples** : 1. The protozoan *Actinosphaerium* (Heliozoa) sends out long, thin pseudopodia within which cytoplasmic particles migrate back and forth. These pseudopodia contain as many as 500 microtubules disposed in a helical configuration. 2. In the protozoan *Nassula*, microtubules drive the food in the gullet. 3. In melanocytes, melanin granules move centrifugally and centripetally with different stimuli. These granules have been observed moving between channels created by the microtubules in the cytoplasmic matrix. 4. In the erythrophores found in fish scales the pigment granules may move at a speed of 25 to 30 μm per second between the microtubules. 5. They have a role in axoplasmic transport of proteins, glycoproteins and enzymes.

MICROFILAMENTS

Thin,solid **microfilaments** of actin protein, ranging between 5 to 7 nm in diameter and indeterminate length, represent the active or motile part of the cytoskeleton. They appear to play major role in cyclosis and amoeboid motion. With high voltage electron microscopy a three-dimensional view of microfilaments has been obtained (*i.e.*, an image of **microtrabecular lattice**). These microfilaments are sensitive to **cytochalasin-B**, an alkaloid that also impairs many cell activities such as beat of heart cell, cell migration, cytokinesis, endocytosis and exocytosis. It is generally assumed that the cytochalasin-B-sensitive microfilaments are the contractile machinery of non-muscle cells.

Distribution

Microfilaments are generally distributed in the cortical regions of the cell just beneath the plasma membrane. In contrast, intermediate filaments and microtubules are found in subcortical and deeper regions of the cell. Microfilaments also extend into cell processes, especially where there is movement. Thus, they are found in the microvilli of the brush border of intestinal epitheliun and in cell types where amoeboid movement and cytoplasmic streaming are prominent.

Chemical Composition

Actin is the main structural protein of microfilaments. The concentration of actin in non-muscle cells is surprisingly high ; it may account up to 10 per cent of total cell protein. It can be extracted and *in vitro* settings will undergo polymerization reactions from G-actin monomer state to F-actin. In fact, the globular (=G actin) – fibrillar (=F-actin) transition is the basis of the classical sol-gel transition in the cytoplasm of moving cells. Further, there are present three types of actins— α , β and γ. The α- form of actin is found in fully mature muscle tissue. The other two forms are more characteristic of non-muscle cells.

Fig. 15.5. Mode of "decoration" of actin filaments by HMM (after Thorpe, 1984).

In non-muscle cells, microfilaments, being of actin composition, can bind myosin (a contractile protein). *In vitro* and *in situ* mircrofilaments can be coated or "decorated" with heavy myosin (HMM) or S_1 heads. This binding results in an arrow-head pattern to the microfilaments in which the arrowheads all point in the same direction (Fig. 15.5). This pattern indicates that microfilaments possess a polarity, a property that is probably crucial to their role in mediating cell movements.

The HMM binding method has become a very useful method for identifying and localizing microfilaments in any type of cell. Intermediate filaments are not decorated by HMM.

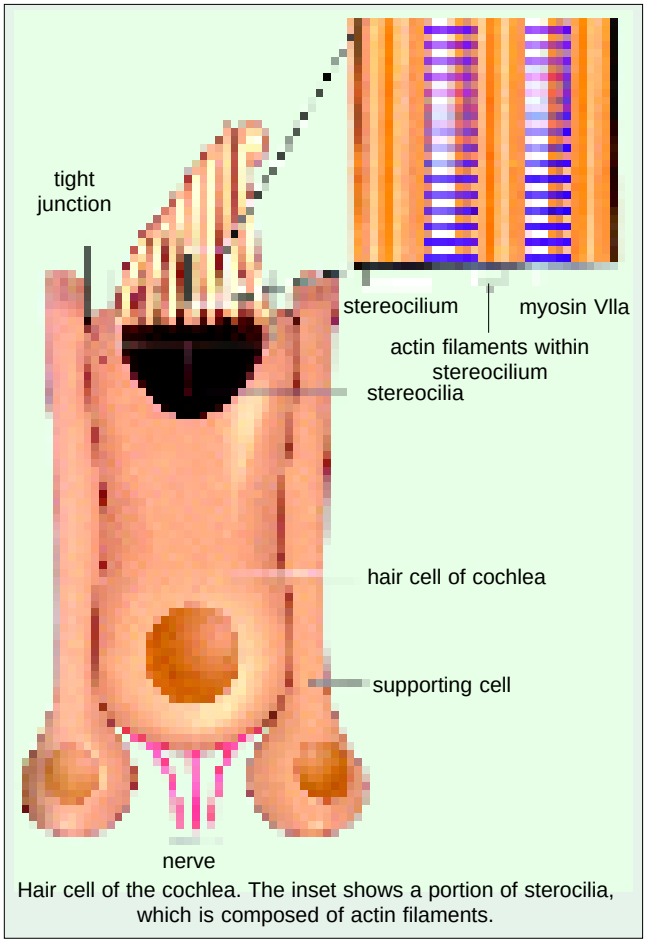
Function

Microfilaments are found to be involved in movement associated with furrow formation in cell division, cytoplasmic streaming in plant cells (*e.g.*, *Nitella* and *Chara*) and cell migration during embryonic development.

INTERMEDIATE FILAMENTS

Intermediate filaments (IFs) are tough and durable protein fibres in the cytoplasm of most higher eukaryotic cells. Constructed like woven ropes, they are typically between 8 nm to 10 nm in diameter, which is "intermediate" between the thin and thick filaments in muscle cells, where they were first described ; their diameter is also between microfilaments (actin filaments) and microtubules. IFs are found resistant to colchicine and cytochalasin B and are sensitive to proteolysis.

In most animal cells IFs form a "basket" around the nucleus and extend out in gentle curving arrays to the cell periphery. IFs are particularly prominent where cells are subjected to mechanical stress, such as in epithelia, where they are linked from cell to cell at desmosomal junctions, along the length of axons, and throughout the cytoplasm of smooth muscle cells. Various names have been attached to the intermediate filaments that have a basis in the cell type in which they are observed. Thus, IFs in epidermal cells are called **tonofilaments**, in nerve cells they are referred to as **neurofilaments** and in neuroglial cells they are designated as **glial filaments**.



Hair cell of the cochlea. The inset shows a portion of sterocilia, which is composed of actin filaments.

In cross-section, intermediate filaments have a tubular appearance. Each tubule appears to be made up of 4 or 5 protofilaments arranged in parallel fashion (**Thorpe**, 1984). IFs are composed of polypeptides of a surprisingly wide range of sizes (from about 40,000 to 130,000 daltons).

Types of intermediate filaments. The intermediate filaments are very heterogeneous from the point of view of their biochemical properties, but by their morphology and localization can be grouped into following four main types (Table 15-1) :

1. Type I IF proteins. They are found primarily in epithelial cells and include two subfamilies of **keratin** (also celled **tono**, **perakeratin** or **cytokeratin**) : acidic keratin and neutral or basic keratin. Keratin filaments are always heteropolymers formed from an equal number of subunits from each of these two keratin subfamilies. The keratins are most complex class of IF proteins,

with at least 19 distinct forms in human epithelia and 8 more in the keratins of hair and nails. Mammalian cytokeratin are α-fibrous proteins that are synthesized in cells of living layers of the epidermis and form the bulk of the dead layers of **stratum corneum**.

2. Type II IF proteins. They include the following four types of polypeptides: vimentin, desmin, synemin and glial fibrillary acidic protein (or glial filaments). **Vimentin** is widely distributed in cells of mesenchymal origin, including fibroblasts, blood vessel endothelial cells and white blood cells, **Desmin** is found in both striated (skeletal and cardiac) and smooth muscle cells. **Glial filaments** occur in some type of glial cells such as astrocytes and some Schwann cells, in the nervous system. **Synemin** is a protein of 230,000 daltons, which is also present in the intermediate filaments of muscle, together with desmin and vimentin. Vimentin and synemin containing IFs can be observed in the chicken erythrocytes.

Each of thse IF proteins tends to assemble spontaneously *in vitro* to form homopolymers and will also co-assemble with the other Types II IF proteins to form **co-polymers** and **heteropolymers**. In fact, co-polymers of vimentin and desmin, or of vimentin and glial fibrillary acidic protein, are found in some type of cells. For example, desmin remains concentrated in the Z-lines and T-tubule system of striated or skeletal system, together with vimentin, synemin and α- actinin. Since desmin links actin to plasma memberane, from this fact the name of desmin has been derived by **Lazarides** and coworkers in 1976 (in Greek desmin means link or bond).

Table 15.1. **Characteristics of four types of intermediate filament proteins (Source : Alberts *et al.*, 1989).**

Types of intermediate filaments	Component polypeptide (mass in daltons)	Cellular location
1. Type I	Acidic keratins (40,000—70,000) Neutral or basic keratins (40,000—70,000)	Epithelial cells and epidermal derivatives such as hair and nail
2. Type II	Vimentin (53,000) Desmin (52,000) Glial fibrillar acidic protein (glial filaments; 45,000) Synemin (230,000)	Many cells of mesenchymal origin Muscle cells Glial cells (astrocytes and some Schwann cells) Muscle cells
3. Type III	Neurofilament proteins (about 130,000, 100,000 and 60,000)	Neurons
4. Type IV	Nuclear lamins A, B and C (65,000—75,000)	Nuclear lamina of all cells

3. Type III IF proteins. These IF proteins assemble into **neurofilaments**, a major cytoskeletal element in nerve axons and dendrites, and consequently are called **neurofilament** proteins. In vertebrates, Type III IFs consist of three distinct polypeptides, the so-called neurofilament triplet.

4. Type IVIF proteins. They are the **nuclear lamins** which form highly organized two dimensional sheets of filaments. These filaments rapidly disassemble and reassemble at specific stage of mitosis.

General Structure of IFs

Despite the large differences in their size, all cytoplasmic IF proteins are encoded by members of the same multigene family. Their amino acid sequences indicate that each IF polypeptide chain contains a homologous central region of about 310 amino acid residues that forms an extended α-helix with three- short— α- helical interruptions (Fig. 15.6).

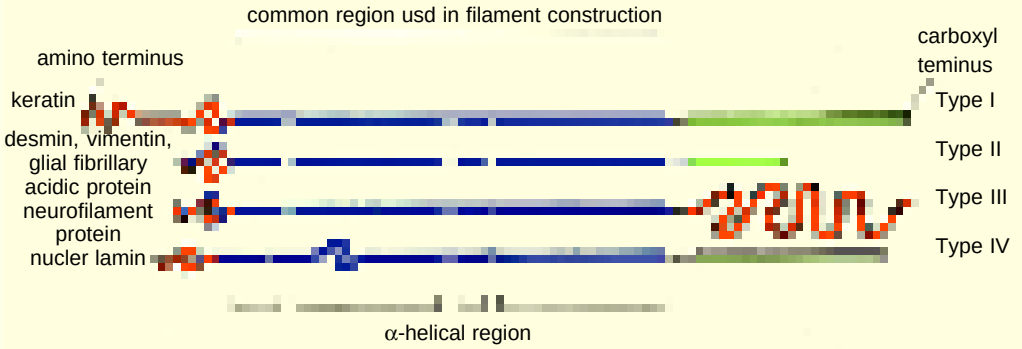


Fig. 15.6. All IF proteins share a similar central region (about 310 amino acid residues) that forms an extended helix with three short interruptions. The amino-terminal and carboxyl-terminal domains are non-helical and vary greatly in size and sequence in different IF proteins (after Alberts *et al*., 1989).

Assembly of IFs

A current model of assembly of a intermediate filament includes the following steps : 1. Two identical **monomers** pair to form a **dimer** in which the conserved helical central regions are aligned in parallel and are wound together into a coiled coil. 2. Two dimers then line up side-by-side to form a 48 nm by 3 nm **protofilament** containing four polypeptide chains. 3.These protofilaments then associate in a staggered manner to form successively larger structures. 4. The final 10 nm diameter of the intermediate filament is thought to be composed of 8-protofilaments (*i.e.*, 32 polypeptide chains) joined end on end to neighbours by staggered overlap to form the long rope-like filaments (Fig.15.7). It is still not known whether IFs are polar structures (like actin and tubulin) or non-polar (like the DNA double helix).

IFs During Mitosis

Mitosis of cultured epithelial cells shows striking changes in intermediate filaments of cytokeratin and vimentin. During prophase the 10 nm filaments unwind into threads of 2 to 4 nm and into spheroidal aggregates containing both types of proteins. At metaphase and anaphase most vimentin and cytokeratin appear as spheroid bodies, while at telophase the filamentous cytoskeleton become gradually reestablished. From these experimental studies, **Franke** (1982) has concluded that the living cells contain factors that promote the reversible disintegration and restoration of intermediate filaments during mitosis.

Functions of IFs

The main function of most intermediate filaments is to provide mechanical support to the cell and its nucleus. IFs in epithelia form a transcellular network that seems designed to resist external forces. The neurofilaments in the nerve cell axons probably resist stresses caused by the motion of the animal, which would otherwise break these long, thin cylinders of cytoplasm. Desmin filaments provide mechanical support for the sarcomeres in muscle cells, and vimentin filaments surround and probably support the large fat droplets in the fat cells.

COMPARISON OF MICROTUBULES, INTERMEDIATE FILAMENTS AND MICROFILAMENTS

The three components of the cytoskeleton, namely microtubules, intermediate filaments and microfilaments have been compared in Table 15-2.

Table 15.2. **Comparison of some properties of microtubules, intermediate filaments and microfilaments (Source : Thorpe, 1984).**

Property	Microtubules	Intermediate filaments	Microfilaments
1. Structure	Hollow with walls made up of 13 protofilaments	Hollow with walls made up of 4 to 5 protofilaments	Solid made up of polymerized actin (F-actin)
2. Diameter (nm)	24 — 25	10	7 — 9
3. Monomer units	α- and β- tubulin	Five types of protein defining five major classes	G-actin
4. ATPase activity	Present in dynein arms	None	None
5. Functions	1. Motility of eukaryotes	1. Integrate contractile units in muscle	1. Muscle contraction
	2. Chromosome movement	2. Cytoskeletal structural function in cytoplasm	2. Cell shape changes
	3. Movements of intracellular materials		3. Protoplasmic streaming
	4. Contribute toward maintaining cell shape		4. Cytokinesis

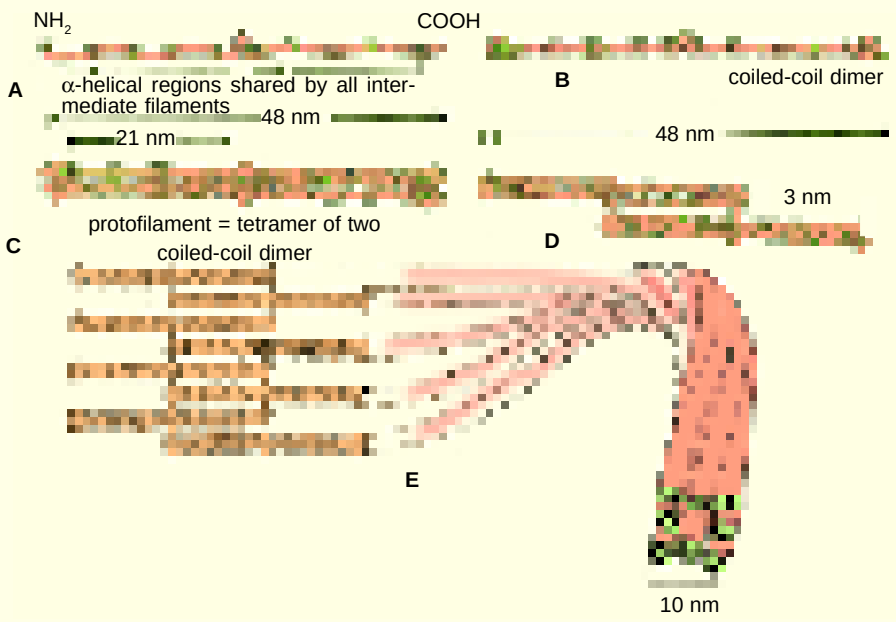


Fig. 15.7. A current model to explain the mode of assembly of a 10-nm thick intermediate filament (after Alberts *et al*., 1989).

REVISION QUESTIONS

1. What is cytoskeleton ? Write a short note on the cytoskeleton
2. What are microtubules ? Describe their structures, assembly, disassembly and functions.
3. What are the intermediate filaments ? Describe their types, structure and cellular functions.
4. Define the term microfilament. Describe the structure and function of microfilament in the cell.
5. Make a comparison of three main components of the cytoskeleton : microtubules, intermediate filaments and microfilaments.